



Article

Supplementary Data for Genetic Engineering of *Agrobacterium* increases Curdlan Production through Increased Expression of the *crdASC* Genes

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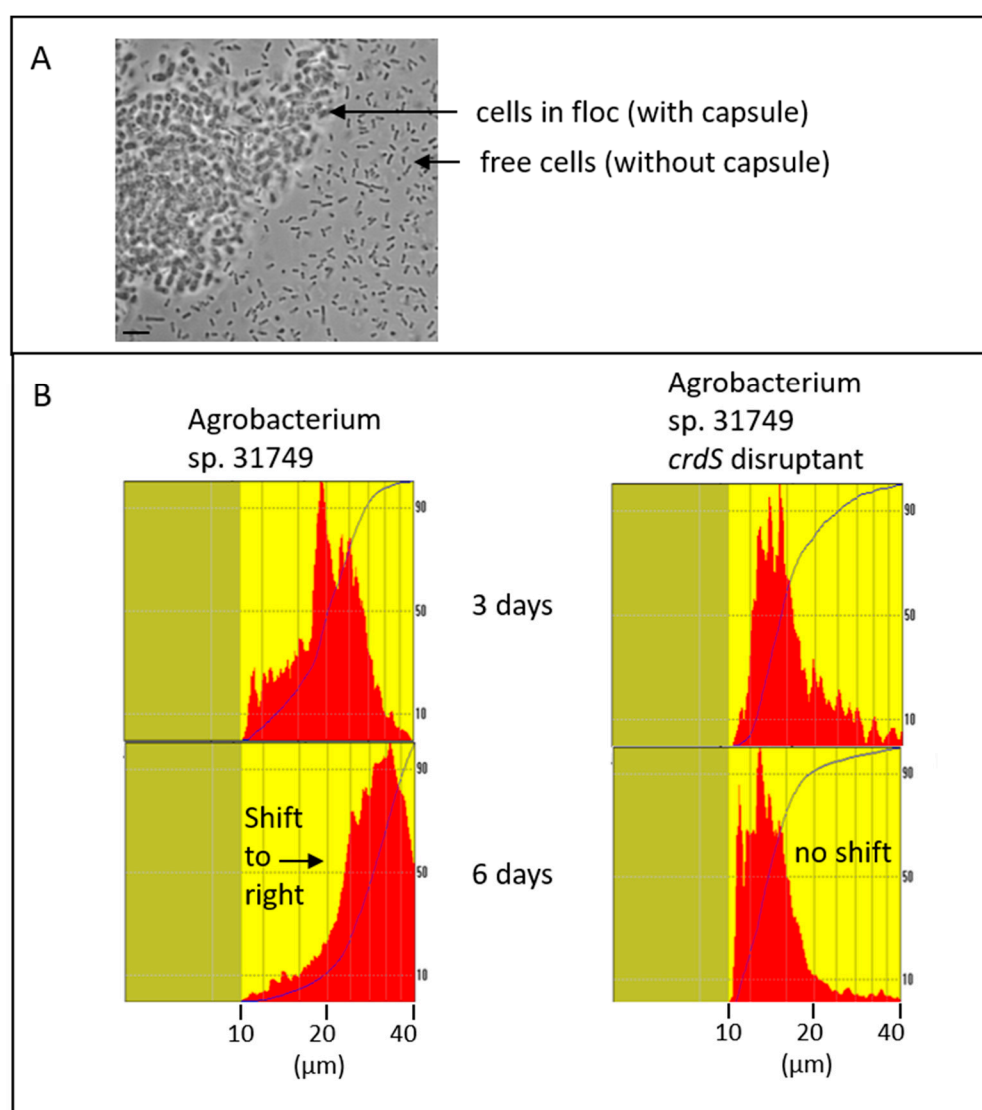


Figure S1. Curdlan promotes cell aggregation. (A) In liquid cultures of *Agrobacterium* defined broth (ADB), encapsulated cells form cell aggregates/flocs, while non-capsulated cells remain free and unattached. Bar represents 5 μm . (B) Liquid cultures were analysed using a particle counter with a 10–40 μm cutoff. The average size of the particles/flocs from the curdlan producing strain were clearly larger and shifted to right (compare 3 days and 6 days), while the particles from the *crdS* disruptant did not.

Supplementary File S1. The DNA sequences of the suicide plasmids used in this study.**primers and plasmids used in this study**

<u>construct name</u>	<u>primer name</u>	<u>primer sequence</u>	<u>application</u>	
pKPcrdA-mV	PcrdA-384H-fwd	cataagcttAGTTTTGGGAAAAAGCAACGG	suicide plasmid 1	
	PcrdA+51X-rev	atgtctagaTCTTACGGATGCGCCTTAT	(PCR for Pcrd)	
pK18-crdA KO	crdA-rbs-sal-fwd	atatgtcgacaacagaggagttgatttcATGctgttcc	suicide plasmid 2	
	crdA+489x-rev	tatatctagagacgagttttgttgcgagccg	(PCR for <i>crdA</i> fragment)	
pK-PphaP-crdA	PphaP-134-H-fwd	catacaagcttGCAACGCAAGGCGTTTCGGG	suicide plasmid 3	
	PphaP-ts-Sal-rev	catagtcgacTCTCCTGCGAGCGGGCTCAT	(PCR for PphaP)	
	crdA-rbs-sal-fwd	atatgtcgacaacagaggagttgatttcATGctgttcc		
	crdA+489x-rev	tatatctagagacgagttttgttgcgagccg	(PCR for <i>crdA</i> fragment)	
pPHU-Pcrd::mVenus	PcrdA-384H-fwd	cataagcttAGTTTTGGGAAAAAGCAACGG	reporter plasmid	
	PcrdA+51X-rev	atgtctagaTCTTACGGATGCGCCTTAT	(PCR for Pcrd)	
pPHU-PphaP::mVenus	P0381-H-fwd2	gataaagcttAATACGGCCATTACACCAAC	reporter plasmid	
	P0381-X-rev2	catatctagaGTCCTGCATCACTTTGCTG	PCR for PphaP, 0381 = <i>phaP</i>)	
	mVenus-E-rev	catgaattcTACTTGTACAGCTCGTCCATGC	reporter, mVenus gene	
	mVenus-X-fwd	tcattctagaATGGTGAGCAAGGGCGAGGAGCT	(PCR for mVenus)	
pK18-crdS KO	CRDS1.2	CGGGATCCTCTAGACGACTTCATCATGGTCTC	suicide plasmid	
	CRDS5.2.2	CGGGATCCTCTAGACCATCAGGCGATATGTCAG	(PCR for <i>crdS</i> fragment)	

DNA sequences relevant for suicide and reporter plasmids

Colour coded key:

Vector sequence (pK18mob2)

crdA fragment

PcrdA fragment

mVenus sequence

PphaP

atg =start codon of *crdA*

various restriction digest sites

> pK18PcrdA-mV, Suicide plasmid 1 (reporter plasmid for 31749)

ACGACGGCCAGTGCC **AAGCTT**AGTTTGGGAAAAAGCAACGGCACTTCCATACCGACTT
 GAGGCGCTAACGCGCTTGCAGTCTGGAATTGACCTACCCGGCGGCTAGGTCTTTGCGACG
 GCATGCCGTCGCCCCCATAAGTTGCATATGCGAAATGGCGAAGCCAGGGTGTGTTTCCA
 AGCACGCCATTTAAAAGGCAATTCTAATATGAAACGATTGAAATTTCTGCTTTCAAATTC
 TCTTCGATGAATTTTCGCGTCCCCAAAATCGTCAGATGCCTATTTGTTGGTCAAAATTGGA
 AAATTAGTTAATGCAATTTTACTATGTTGCGGCGCACAAGAACTTCATTACCATTTCAA
 TACTGCGGGAGGAACGGTTTCTCAACAACAGAGGAGTTGATTT**atg**CTGTTCCGCAATAA
 GCCTGACGCAAAATATAAAGGCGCATCCGTAAGA **TCTAGA**ATGGTGAGCAAGGGCGAG
 GAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCA
 CAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGA
 AGCTGATCTGCACCACCGCAAGCTGCCCCGTGCCCTGGCCACCCTCGTGACCACCCTGG
 GCTACGGCCTGCAGTGCTTCGCCCCGTACCCCGACCACATGAAGCAGCACGACTTCTTCA
 AGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGC
 AACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCG
 AGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTA
 CAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAG
 GCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTA
 CCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGA
 GCTACCAGTCCAAACTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTCTGCTG
 GAGTTCGTGACCGCCGCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA**GAATT**
GTA

> pK18PcrdA-mV, Suicide plasmid 1 (reporter plasmid for C58)

ACGACGGCCAGTGCC **AAGCTT**AGTTTGGGAAAAAGCAACGGCACTTCCATACCGACTT
 GAGGCGCTAACGCGCTTGCAGTCTGGAATTGACCTACCCGGCGGCGAGGCCTTTGCGAC
 GGCATGCCGTCGCCCCCATAAGTTGCATATGCGAAATGGCGAAGCCAGGGTGTGTTTCC
 AAGCACGCCATTTAAAAGGCAATTCTAATATGAAACGATTGAAATTTCTGCTTTCAAATT
 CTCTTCGATGAATTTTCGCGTCCCCAAAATCGTCAGATGCCTATTTGTTGGTCAAAATTGG
 AAAATTAGTTAATGCAATTTTACTATGTTGCGGCGCACAAGAACTTCATTACCATTTCA
 ATaCTGCGGGAGGAACGGTTTCTCAACAACAGAGGAGTTGATTT**atg**CTGTTCCGCAATA
 AGCCTGACGCAAAATATAAAGGCGCATCCGTAAGA **TCTAGA**ATGGTGAGCAAGGGCGA
 GGAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC
 ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTG
 AAGCTGATCTGCACCACCGCAAGCTGCCCCGTGCCCTGGCCACCCTCGTGACCACCCTG
 GGCTACGGCCTGCAGTGCTTCGCCCCGTACCCCGACCACATGAAGCAGCACGACTTCTTC
 AAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGG
 CAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCG
 AGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTA
 CAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAG
 GCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTA
 CCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGA
 GCTACCAGTCCAAACTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTCTGCTG

GAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA GAATT
CGTA

>pK18-crdA KO, Suicide plasmid 2 (KO plasmid for C58)

ACGACGGCCAGTGCC AAGCTT GCATGCCTGCAG GTCGAC AACAGAGGAGTTGATTTCatg
CTGTTCCGCAATAAGCCTGACGCAAAATATAAAGGCGCATCCGTAAGATTAGCCTCCTAT
ACTGTTCTCGGTGTGGTGGCGACGGTGTCTTCTTCTCCGTGTTCCGCCTGTATTACCTC
TCAAACGAGCCGTGCTCTGATCAATGCCCCATTATTCCCTTGACGACGCCGATCAGCGG
CGTTGTGAAAACATCGAACGACAGGGGCGGTGCCACCATCGAGAACGACAAGGTGGAC
AATACAACGCTGATCGGCCTGAAGGTTCAACTGGCGGCGCTTGACAATGAGATGCGCCA
GAAGAACTCGATCGTTTCGGACTATGCCTCGCGGATCGACGACCTGAACAGGGATCTTTC
CAGCCAGCAGGCGGCTTTGCTGCTGCGGACAGAGTCCGATCTCGAGGCTGCAGAGGCGG
CCTTGAGATGGTTACCTATTCCACGCGGATCGAAAAGCCGAAGCGGCTCGCAAACAA
AAACTCGTCTCTAGAGGATCCCCGGGTACCGAGCTC GAATTC GTA

>pK18-PphaP-crdA, Suicide plasmid 3 (over-expression plasmid for 31749)

AAGCTT GCAACGCAAGGCGTTTCGGGCCAGGTGCGAAAAAATGCTGCGGTGCAGAATGA
GCGGTTGATTTCTGCGGCGCAGCATTATATCCTTGTCACAGGATAACCGGGCATGAGC
CCGCTCGCAGGAGAGTCTGAC AACAGAGGAGTTGATTTCatgCTGTTCCGCAATAAGCCTG
ACGCAAAATATAAAGGCGCATCCGTAAGATTAGCCTCCTATACTGTTCTCGGTGTGGTGG
CGACGGTGTCTTCTTCTCCGTGTTCCGCCTGTATTACCTCTCAAACGAGCCGTGCTCTG
ATCAATGCCCCATTATTCCCTTGACGACGCCGATCAGCGGCGTTGTGAAAACATCGAAC
GACAGGGGCGGTGCCACCATCGAGAACGACAAGGTGGACAATACAACGCTGATCGGCC
TGAAGGTTCAACTGGCGGCGCTTGACAATGAGATGCGCCAGAAGAACTCGATCGTTTCG
GACTATGCCTCGCGGATCGACGACCTGAACAGGGATCTTTCAGCCAGCAGGCGGCTTT
GCTGCTGCGGACAGAGTCCGATCTCGAGGCTGCAGAGGCGGCTTGCAGATGGTTACCT
ATTCCACGCGGATCGAAAAGCCGAAGCGGCTCGAAACAAAAACTCGTCTCTAGAGG
ATCCCCGGGTACCGAGCTC GAATTC GTA

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